Available online www.jocpr.com

Journal of Chemical and Pharmaceutical Research, 2016, 8(12):173-179

Research Article

ISSN : 0975-7384
CODEN(USA) : JCPRC5

Antibacterial Effects of Different Extracts of *Crocus Sativus* Linn Stigma on Several Oral Microorganisms

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ABSTRACT

Background: Antimicrobial therapy against oral pathogens is a common way to prevent or treat mouth diseases. Because of increasing resistance to synthetic medicine, herbal drugs are being used more and more.
Material and Methods: Hydroalcoholic extract of powdered stigma from saffron flower was provided and used to prepare fractions in petroleum-ether, chloroform, and ethanol solvents respectively. Growth inhibition zone, MIC and MBC of the mentioned fractions were determined against Strep. mutans, Strep. sanguinis, Strep. salivarius, Strep. sobrinus, and E. faecalis and compared to those of chlorhexidine and ampicillin.
Results: The hydroalcoholic extract, and ethanolic and chloroformic fractions of crocus sativus linn had antimicrobial effects, despite its petroleum etheric fraction and distillate. The best effect of hydroalcoholic extract was on Strep. sanguinis with the MIC and MBC of 31.25 mg/ml both. Maximum inhibitory effect of ethanol fraction was on Strep. sanguinis with MIC and MBC of 7.81 and 15.62 mg/ml respectively. Chloroform fraction presented most effect on Strep. sanguinis presenting MIC and MBC of 7.8 mg/ml.
Conclusion: Crocus sativus linn contains agents with antimicrobial properties against some oral pathogens, particularly streptococcus sanguinis. It may be used to prepare antimicrobial drugs and mouthwashes in the future.

Keywords: Saffron; Antimicrobial activity; Oral pathogens; Hydroalcoholic extract; Soluble fractions

INTRODUCTION

Dental caries, periodontal disease, and opportunistic infections, common diseases in oral cavity, are caused by the accumulation of pathogenic microorganisms, inappropriate nutrition, and poor oral hygiene [1-4]. Oral streptococci are the first isolated species participating in the formation of dental plaque and development of caries [5-7]. Measures taken to clinically inhibit plaque accumulation, including tooth brushing, dental flossing, and use of mouthwashes (as an adjunct), are highly effective, prevent gingivitis and decrease the number of microorganisms [8, 9]. Chlorhexidine is one of the most widely used antimicrobial agents. However, it has side effects such as altered sense of taste and staining of the teeth and restorations [10-13].
Herbal medicine has a long history. Many people in developing countries strongly believe in the benefit of herbal medications for primary care [14, 15]. While synthetic drugs have side effects and there is an increasing number of resistant microorganisms to them, researchers are becoming more and more interested in finding alternative herbal medications and their active components [16-23].

Saffron with the botanical name of Crocus sativus linn is a small perennial flowering plant from the family of Iridaceae which grows up to 10-30 centimeters tall. It has a bulbous corm which is covered by a thin brown sheath and multiple long and linear leaves arise from that. From within the leaves, a stem comes out which ends in 1 to 3 flowers. The attractive flowers consist of 6 lilac (in certain types purplish or rose-coloured) petals, three stamens, and a style ending in three red-orange stigmas. The terminal end of style, the three-pronged stigma, is the part being used and called saffron, which is fragrant and a little bitter. Geographic distribution of saffron in Iran includes Khorassan province (Ghaenat, Birjand, and Gonabad), Yazd, Kerman, Guilan, and Mazandaran [24].

Saffron is not only a highly used flavoring in cuisines, but also has multiple pharmacological effects and is considered to be a potent drug [25]. In traditional medicine saffron and its extract have been used to facilitate digestion, stimulate appetite, tranquilize, and to treat liver diseases, spasm, pain of tooth or gingiva, rhinitis, pharyngitis, insomnia, depression, seizure, irregular menstruation, cough, asthma, bronchitis, fever, vomiting, scarlet fever, urinary infections, dysentery, cold, cardiovascular disorders, and cancer. In Ayurvedic medicine (a type of traditional Hindu medicine) saffron is used to enhance body resistance against stresses like trauma, anxiety, and fatigue [26-30].

Due to modern researches saffron has effects such as antitumor, antioxidant, protection of cells, analgesic, anti-inflammatory, anticonvulsant, antidepressant, reduction of opioid withdrawal signs, improvement in memory and learning ability, antibacterial and effects on respiratory system, digestion, immune system, and eye [30, 29, 31-37].

Crocus sativus linn has recently been studied for antimicrobial effects. Its effect on some microorganisms like salmonella [38, 39], shigella dysenteria, methicillin resistant staph aureus [39], Escherichia-coli [39, 40], Pseudomonas aeruginosa [39, 41], Klebsiella sp [39, 41] bacillus subtillis [41, 39], Yersinia enterecolitica, Proteus vulgaris, Bacillus cereus, Staphylococcus aureus, Micrococcus luteus and Candida albicans [41], fungi [42], and Brucella melitensis [43] has been shown.

Vahidi et al. found strong antimicrobial effects of ethyl-acetate extract of saffron against several microorganisms [44].

Also, Mudasir et al. showed that various extracts of saffron had antimicrobial effects on some gram positive and gram negative microbes [45].

However, these studies are too few and mostly lack clear description of applied microbial methodology. None is conducted on oral pathogens.

Considering high prevalence of oral and dental diseases caused by pathogens and the recent interest in medicinal plants, this study conducted to assess the effect of various extracts of saffron plant on Strep. mutans, Strep. sanguinis, Strep. salivarius, Strep. sobrinus, and E. faecalis in vitro.

MATERIALS AND METHODS

Saffron flowers were obtained from the last harvest in Ghaenat city and its purity was confirmed in a pharmacognosy laboratory. The plant was powdered in a porcelain mortar with pestle. Before extraction, dried stigma of saffron was totally grinded. Crocus sativus linn extract was prepared by maceration method. Powdered saffron was precisely measured by a digital scale and poured into an Erlenmeyer flask. Hydroalcoholic solvent (50% water and 50% ethanol) was also added. The Erlenmeyer flasks were capped with aluminum foil and stored in the dark for 10 days. Next, the flasks were placed on a shaker (GFL 3017) operating at 90 rpm for 24 hours. The solutions were then paper filtered. The filtered solution was poured into a sterile glass container and capped by aluminum foil. A few holes were perforated in the foil and the glass container was placed in Bain-Marie at 90∘C to dry. The dried extract was precisely weighed, labeled, and refrigerated [46].

Then to extract probable antimicrobial materials of the plant, and to find the degree of its polarity, part of the extract made was powdered and solved respectively in petroleum-ether, chloroform, and ethanol. Each solution after 48 hours on the shaker (GFL, 3017) was filtered and then dried in Bain-Marie at 90∘C. The filtered material was added to the next solvent. The result was three different fractions of saffron extract. Dried extracts were precisely weighed, labeled, and refrigerated [46].

To make saffron distillate, the weighed powder was put in collenger and distilled water was poured on it. Then it was heated with an electric heater to boil. The device was turned off after a few hours. The distilled liquid is the saffron distillate. The distillate's container was capped with aluminum foil to protect from vaporization or deterioration. Then it was refrigerated [46].

Activation of microorganism

Standard strains of Strep. mutans ("ATCC" 35668, "PTCC" 1683), Strep. sanguinis ("ATCC" 10556, "PTCC" 1449), Strep. salivarius ("ATCC" 9222, "PTCC" 1448), Strep. sobrinus ("ATCC" 27607 and "PTCC" 1601), and E. faecalis ("ATCC" 11700, "PTCC" 1393) were provided in lyophilized form from the Persian Type Culture Collection center. Bacteria became activated by inoculation in the brain heart infusion agar (BHIA,

Merck, Germany) culture medium, then 24 hours of incubation at 37∘C. To prepare microbial suspension, a 24-hour culture was used. The concentration of microorganisms in the microbial suspension was adjusted to 0.5 McFarland standard by a spectrophotometer at wavelength of 625 nm. (McFarland standard is a chemical solution with turbidity comparable to that of microbial suspension. Using this suspension, number of bacteria per each milliliter of the suspension is set to be 1.5×10^8 CFU/mL) [47].

Assessment of antimicrobial effects

First, the antimicrobial effect of extracts was assessed by the cup-plate technique. 500 µL of each microbial suspension with 0.5 McFarland standard concentration was cultured in BHIA (swabbed on the plate). Then, holes of 8mm diameter were created on the agar surface. Various concentrations of the extracts were prepared by serial dilution (dilution by one-half) using sterile distilled water solvent; 100 µL of each extract with specific concentration was poured into each well. The plates were incubated at 37∘C (Memmert, Germany) for 24 hours. The diameter of the growth inhibition zone measured in millimeters for three times and the mean was calculated for each concentrations of the extract [47].

Minimum inhibitory concentration

The MIC is defined as the lowest concentration of an antimicrobial agent that inhibits the growth of a microorganism (0.5 McFarland standard in this study). That is the minimum concentration of the extract which completely inhibits visible growth and proliferation of bacteria compared to the negative control group. To determine MIC, macrodilution method according, i.e. the standard technique described by the clinical and laboratory standards institute (2012), was used. Different concentrations of extracts were prepared by serial dilution (dilution by one-half) in BHI broth medium. Using this medium, the 0.5 McFarland standard suspension was diluted 1:150 to reach a bacterial count of 1×10^6 CFU/mL. Microbial suspension was then diluted by one-half using the culture medium and 1mL of it was added to the tubes of serially diluted extract. The negative control tube contained only the culture medium and extract without any microbial suspension. The positive control tube contained culture medium and microbial suspension without any extract. After 24 hours of incubation at 37∘C, growth and proliferation of each microorganism were evaluated and the MIC value of the extracts was determined. This test was done in triplicate for each microorganism [47].

Minimum bactericidal concentration

After determination of MIC, 20 µL of the suspension in the tube with MIC of the extract and the tubes of no bacterial growth were cultured on plates containing BHIA. After 24 hours of incubation at 37∘C, the plates were assessed for growth of microorganisms. The concentration with no bacterial growth was determined as MBC. This test was repeated three times for each microorganism [47]. Furthermore, the effect of 0.2% CHX (ShahrDaru, Iran) and ampicillin on the microorganisms was evaluated using the cup-plate technique and the MIC and MBC values of both for each microorganism were also determined.

Statistical analysis

The tests were repeated in triplicate and the mean and standard deviation (SD) of growth inhibition zone diameter in cup-plate technique as well as the MIC and MBC of the extracts, CHX, and ampicillin were determined.

RESULTS

The hydroalcoholic extract of crocus sativus linn in concentrations of 500 mg/ml and 1000 mg/ml showed inhibitory effects on the growth and proliferation of streptococcus mutans, streptococcus sanguinis, and streptococcus sobrinus in cup-plate method. No inhibition zone was detected for streptococcus salivarius and enterococcus faecalis. The mean diameter of the growth inhibition zone due to the effect of hydroalcoholic extract of C. sativus linn on different microorganisms is shown in Table 1

The MIC and MBC of hydroalcoholic extract of C. sativus linn were determined using serial dilution method. Due to the results, they changed between 31.25 and 500 mg/ml. The best effect of hydroalcoholic extract was on streptococcus sanguinis with the MIC and MBC of 31.25 mg/ml both.

The MIC and MBC values of different fractions of saffron for each microorganism are presented in Tables 2 and 3.

Table 1: **The mean and SD of the diameter of growth inhibition zone (mm) due to the effect of the hydroalcoholic extract of C. sativus linn petal on different microorganisms. N.S.: not seen**

Concentration (mg/ml)	Microorganism				
	S.mutans	***S.sanguinis***	***S.salivarius***	***S.sobrinus***	***E.faecalis***
1000	15±0	15±0	N.S.	14 ±0	N.S.
500	12±0	12±0	N.S.	11±0	N.S.
250	N.S.	N.S.	N.S.	N.S.	N.S.
125	N.S.	N.S.	N.S.	N.S.	N.S.
62.5	N.S.	N.S.	N.S.	N.S.	N.S.
31.25	N.S.	N.S.	N.S.	N.S.	N.S.

Table 2: The MIC of different extracts of C. sativus linn, CHX, and Ampicillin for different microorganisms

Microorganism	Type of saffron extract			CHX 0.2% (µg/ml)	Ampicillin (µg/ml)
	Hydroalcoholic (mg/ml)	Ethanolic (mg/ml)	Chloroformic (mg/ml)		
S. mutans	62.5	31.25	31.25	0.09	0.06
S. sanguinis	31.25	7.81	7.81	0.02	0.015
S. salivarius	62.5	31.25	31.25	0.78	0.125
S. sobrinus	62.5	62.5	31.25	0.04	0.125
E. faecalis	>500	250	125	6.25	2

Table 3: The MBC of different extracts of C. sativus linn, CHX, and Ampicillin for different microorganisms

Microorganism	Type of saffron extract			CHX 0.2% (µg/ml)	Ampicillin (µg/ml)
	Hydroalcoholic (mg/ml)	Ethanolic (mg/ml)	Chloroformic (mg/ml)		
S. mutans	125	125	31.25	0.09	0.125
S. sanguinis	31.25	15.62	7.8	0.012	0.03
S. salivarius	125	125	62.5	0.39	0.125
S. sobrinus	62.5	125	31.25	0.02	0.25
E. faecalis	>500	500	50	3.125	4

The cup-plate method showed inhibitory effects of ethanol and chloroform fractions in 500 mg/ml concentration, and no inhibition zone was seen for petroleum-ether extract.

The strongest inhibitory effect of ethanol fraction of C. sativus Linn was on streptococcus sanguinis with MIC and MBC of 7.81 and 15.62 mg/ml respectively. Minimum inhibitory concentration and minimum bactericidal concentration of ethanol fraction are shown in Tables 2 and 3.

For chloroform fraction the range of MIC and MBC were 7.8-125 and 7.8-500 mg/ml respectively. This fraction presented greatest effect on streptococcus sanguinis presenting MIC and MBC of 7.8 mg/ml. Minimum inhibitory concentration and minimum bactericidal concentration of chloroformic fraction are shown in Tables 2 and 3.

Chlorhexidine 0.2% had antimicrobial effect on all five bacteria (highest effect on streptococcus sanguinis) with cup-plate method. The mean diameter of the growth inhibition zone due to the effect of chlorhexidine on different microorganisms is shown in Table 4.

Table 4: The mean and SD of the diameter of growth inhibition zone (mm) due to the effect of 0.2%CHX on the five bacterial strains

Concentration (µg/ml)	Microorganism				
	S.mutans	***S.sanguinis***	***S.salivarius***	***S.sobrinus***	***E.faecalis***
100	>30±0	>30±0	>30±0	>30±0	23.5±0.41
50	>30±0	>30±0	>30±0	>30±0	21±0
25	>30±0	>30±0	>30±0	>30±0	18.5±0.41
12.5	>30±0	>30±0	>30±0	>30±0	16.5±0.41
6.25	>30±0	>30±0	>30±0	>30±0	14±0
3.125	>30±0	>30±0	>30±0	>30±0	12±0

Broth dilution method resulted in the range of 0.012 to 3.125 for MIC of chlorhexidine 0.2%. Minimum inhibitory concentration and minimum bactericidal concentration of chlorhexidine are shown in Tables 2 and 3.

The cup-plate method showed that ampicillin had antimicrobial effect on all five bacterial strains. The mean of the diameter of growth inhibition zone due to the effect of ampicillin on different microorganisms are shown in Table 5.

Table 5: The mean and SD of the diameter of growth inhibition zone (mm) due to the effect of ampicillin on the five bacterial strains
N.S.: not seen

Concentration (µg/ml)	Microorganism				
	S.mutans	***S.sanguinis***	***S.salivarius***	***S.sobrinus***	***E.faecalis***
16	>30±0	>30±0	30±0	>30±0	18±0
8	>30±0	>30±0	26.5±0.41	28±0	16±0
4	30±0	30.5±0.41	22.5±0.41	24.5±0.41	14±0
2	24.5±0.41	25±0	18±0	16±0	11.5±0.41
1	18±0	20±0	15±0	12±0	N.S.
0.5	14.5±0.41	>30±0	N.S.	N.S.	N.S.

Broth dilution method resulted in the range of 0.015 to 2 for MIC of ampicillin. Tables 2 and 3 show the minimum inhibitory concentration and minimum bactericidal concentration of ampicillin for the five bacterial strains; the cup-plate method for saffron distillate didn't show inhibition zone on any of the studied microorganisms.

DISCUSSION

This study was the first to experimentally assess the antimicrobial effect of hydroalcoholic extract of *C. sativus* petal on *Strep. mutans*, *Strep. sanguinis*, *Strep. salivarius*, *Strep. sobrinus*, and *E. faecalis* in vitro using the cup-plate method. The MIC, MBC, and growth inhibition zone diameter values of the extract for different microorganisms were also calculated and compared to CHX and ampicillin.

Based on the results, the hydroalcoholic extract and ethanolic and chloroformic fractions of crocus sativus linn have antimicrobial effects, but petroleum etheric fraction and distillate of c. sativus linn don't. Moreover, none of the methods showed significant influence on streptococcus salivarius and enterococcus faecalis.

Infiltration rate of an antimicrobial agent in agar depends on its nature and is an influencing factor on the results of cup-plate method. This method is used to determine if a microorganism is sensitive to the antimicrobial agent or not. On the other hand, broth dilution method for detection of MIC is more accurate [46]. Then broth dilution method is more reliable.

In broth dilution method, extracts of ethanolic and chloroformic fractions showed more inhibitory effect than hydroalcoholic extract. Evaluating MIC and MBC resulted in best effect of all three extracts on streptococcus sanguinis and the least effect on enterococcus faecalis. Chlorhexidine and ampicillin also had the most and the least effects respectively on streptococcus sanguinis and enterococcus faecalis. This might be caused by higher sensitivity in Strep. sanguinis and more resistance in E. faecalis.

In a study by Sengul et al. about antimicrobial effects of saffron, water extract of the stigma affected only two species from the 32 assessed, while alcoholic extract of c. sativus linn had effect on 16 species. It means alcohol solvent releases more antimicrobial materials from this plant compared to water solvent [33].

Mudasir et al. assessed antimicrobial effect of stamen and stigma of the Keshmir saffron against three gram-positive microbes (bacillus cereus, streptococcus aureus, and clostridium perfringens) and three gram-negative microbes (E-coli, pseudomonas aeruginosa, and klebsiella pneumonia). The results revealed more inhibitory effect of petroleum-ether, methanol, and ethanol extracts of stamen in lower concentrations on Klebsiella compared to penicillin. Ethanolic extract of stamen had inhibition on bacillus cereus more than gentamycin. The extract of stamen had more antimicrobial activity against different pathogens in comparison with stigma. Both were declared as new, safe, and natural materials for medical, cosmetic and alimentary purposes [45].

A study by Acar et al. revealed that ethyl acetate and methanol extracts of crocus species, including sativus, had inhibitory effects on E-coli, Pseudomonas aeruginosa, Klebsiella pneumoniae, Yersinia enterecolitica, Proteus vulgaris, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus, Micrococcus luteus and Candida albicans. According to Acar, ethyl acetate extracts were more influential than methanol extracts [41]; the same as Vahidi's study in which ethyl acetate showed better results than ethanol and petroleum ether [44]. Vahidi et al. evaluated antimicrobial effect of different parts (stigma, stamen, and colora) of saffron plant on micrococcus lotus, streptococcus epidermis, staphylococcus aureus, and E-coli bacteria, and on candida albicans, aspergillus niger, and cladosporium sp fungi. The results showed strong antimicrobial and antifungal effects of ethyl-acetate extract of various parts. Stigma had more antimicrobial effect than stamen and stamen had more antifungal effect than stigma.

Jastaniah showed in a study that c. sativus had antimicrobial effect on Methicillin-resistant *Staphylococcus aureus*, and that methanol extract was the strongest between methanol, ethanol, n-butanol, chloroform, ethyl acetate or water extracts [39].

Sengul et al. also revealed that methanol extract of crocus sativus has antimicrobial effects (more than aqueous extract) against specific microorganisms, 13 out of 32 in their study [33].

One important factor affecting the MIC is the difference in the composition of extracts. The composition of extract is influenced by the geographical location of the plant, season of harvesting, age of plant, growth stage, method of drying, and extraction technique. Also, extracts of various parts of the plant have different levels of

antimicrobial activity and bacteria any of them has different sensitivity to different extracts. Also, isolated components of an extract show greater antimicrobial effects than the extract itself [48]. The stigma of c. sativus linn comprises the antimicrobial agents of Linalool, Myricetin, Borneol, Kaempferol, Lauric acid, Limonene, Pinene, Quercetin, Oleanolic acid, carotenoids (crocin, crocetin, a-carotenolicopen), monoterpenaldehydes (*picrocrocin* and *safranal*), and flavonoids [31]. It also contains dimethylcrocetin carotenoid [49] and hexadecanoic acid [42]. Razzaghi et al. revealed inhibitory effect of safranal in saffron on E-coli, staphylococcus aureus, and pseudomonas aeruginosa [50].

De Monte et al. showed effects of safranal and its derivatives on helicobacter pylori, malaria and leishmanial [51].

Pintado et al. showed the bactericidal effect of safranal and crocin on salmonella enterica, Escherichia coli and Staphylococcus aureus [52].

Distillate of saffron releases some parts of the plant which had no inhibitory effect on our test microorganisms. Also, the fraction solved in petroleum ether doesn't show antimicrobial properties, and only water-alcohol and chloroform soluble fractions have antimicrobial effects.

Chlorhexidine mouthwash has extensive antimicrobial activity and is more effective on Gram-positive than Gram-negative bacteria. It is known as the most effective mouthwash and the gold standard of antibacterial activity [53]. The MIC and MBC values and inhibitory effects for CHX obtained in this study were in accordance with the results of specific microbiological assessments on CHX and ampicillin, which confirmed the accuracy and precision of the laboratory phases in our study.

One limitation of our study was that we tested the saffron flowers only harvested at a specific time and from a specific location. In addition, standardized microbial strains were used and microorganisms originally from the oral cavity were not assessed. Hence, the results were limited to standard strains in the laboratory setting. Further studies are required to confirm the results in oral biological environment. Also, future studies must focus on the isolated constituents of different fractions. If the effects of these constituents on other oral pathogens, such as candida albicans, are confirmed, they could be used in the form of pure extract, mouth rinse, or other antimicrobial products in clinical trials.

CONCLUSION

Within the limitations of this study, it can be concluded that crocus sativus linn contains agents which show antimicrobial effects. Among different extracts of saffron, hydroalcoholic extract, and ethanolic and chloroformic fractions of saffron showed significant effects against examined bacteria, particularly streptococcus sanguinis. However, chlorhexidine shows wider spectrum of antimicrobial effects with lower concentrations. So, further studies are needed to be conducted to search for more efficacious methods of extracting antimicrobial agents of saffron.

REFERENCES

[1] A Dziedzic; R Kubina; RD Wojtyczka; A Kabala-Dzik; M Tanasiewicz; T Morawiec. Evidence based complementary alternative medicine: eCAM 2013, **2013**, 681891.
[2] WP Holbrook; MO Magnusdottir. *J oral microbial*, **2012**, 4.
[3] JR Pires; C Rossa Junior; AC Pizzolitto. *Braz oral res* **2007**, 21(4), 342-7.
[4] A Derks; J Frencken; E Bronkhorst; AM Kuijpers-Jagtman; C Katsaros. *Am j orthod dentofac orthop,* **2008,** 133(3), 435-439.
[5] H Semyari; P Owlia; S Farhadi; SM Tabrizi. *J Microbiol Antimicrob*, **2011**, 3(5), 126-129.
[6] JD Hillman; SS Socransky; M Shivers. *Arch oral boil*, **1985**, 30(11-12), 791-795.
[7] J Clarke. Br *J Exp Pathol* **1924**, 5(3), 141-147.
[8] EA Palmer; A Vo; SB Hiles; P Peirano; S Chaudhry; A Trevor; I Kasimi; J Pollard; C Kyles; M Leo; B Wilmot; J Engle; J Peterson; T Maier; CA Machida. *J oral microbial*, **2012,** 4.
[9] RP Teles; FR Teles. *Braz oral res* **2009**, 23(Suppl 1), 39-48.
[10] JC Gunsolley. *J dent* **2010,** 38(Suppl 1), S6-10.
[11] CA Gurgan; E Zaim; I Bakirsoy; E Soykan. *J periodontal*, **2006,** 77(3), 370-84.
[12] AM Abed; A Bateni; A Rabiee; B Poormoradi. *J Isfahan Dent*, **2012,** 7(5), 843-61.
[13] LS Lakade; P Shah; D Shirol. *J Indian Soc Pedod Prev Dent*, **2014,** 32(2), 91.
[14] EA Palombo. Evidence-based complementary and alternative medicine : eCAM 2011, **2011,** 680354.
[15]DP Mohapatra; V Thakur; SK Brar. *Biotechnol res int*, 2011, **2011,** 917505.
[16] S Vahabi; E Najafi; S Alizadeh. *J Med Plants Res*, **2011**, 5(19), 4870-4878.
[17] JB Taheri; S Azimi; N Rafieian; HA Zanjani. *Int dental j*, **2011,** 61(6), 287-96.
[18] N Nagappan; J John. *J Dent Med Sci*, **2012,** 2(4), 5-10.
[19] I Ahmad; AZ Beg. *J ethnopharmacol*, **2001,** 74(2), 113-23.

[20] Z Dalirsani; M Aghazadeh; M Adibpour; M Amirchaghm; A Pakfetrat; PM Mozaffari; M Mehdipour; AT Zenooz. *J Appl Sci*, **2011**, 11(5), 878-882.
[21] IA Freires; C Denny; B Benso; SM de Alencar; PL Rosalen. Molecules (Basel, Switzerland) **2015,** 20(4), 7329-58.
[22] D Gupta; S Nayan; HK Tippanawar; GI Patil; A Jain; RK Momin; RK Gupta. *Pharmacognosy res*, 2015, 7(3), 277-281.
[23] F Hajifattahi; E Moravej-Salehi; M Taheri; A Mahboubi; M Kamalinejad. *Int j biomaterials*, 2016, **2016**, 8098943.
[24] MH Salehi-Surmaghi. *Medicinal plants and phytotherapy*, the World of Nutrition Publications, Tehran, Iran, 2006.
[25] SK Verma; A Bordia. *Indian j medical sci*, **1998,** 52(5), 205-207.
[26] B Javadi; A Sahebkar; SA Emami. *Iranian j basic medical sci*, **2013,** 16(1), 1-11.
[27] S Kianbakht; A Ghazavi. *Ethnopharmacologia*, **2005,** 36, 78-83.
[28] C Licón; M Carmona; S Llorens; MI Berruga; GL Alonso. *Functional Plant Sci Biotechnol*, **2010,** 4, 64-73.
[29] V Bhargava. *Int J Pharmacy Pharmaceutical Sci*, **2011,** 3(3), 22-26.
[30] A Kyriakoudi; A Ordoudi; M Roldán-Medina; Z Tsimidou. *Austin J Nutri Food Sci*, **2015**, 3(1), 1059.
[31] S Kianbakht. *J Med Plants*, **2008,** 4(28), 1-27.
[32] M Khalili; F Hamzeh. *Iranian biomed j*, **2010,** 14(1-2), 59-65.
[33] M Sengul; H Yildiz; N Gungor; B Cetin; Z Eser; S Ercisli. *Pak j pharm sci*, **2009**, 22(1), 102-6.
[34] E Christodoulou; NP Kadoglou; N Kostomitsopoulos; G Valsami. *J pharm pharmacol* **2015**, 67(12), 1634-49.
[35] SA Goli; F Mokhtari; M Rahimmalek. *J Agr Sci*, **2012,** 4(10), 175.
[36] H Hosseinzadeh; Z Jahanian. *Phytotherapy res*, *PTR* **2010**, 24(5), 726-30.
[37] HA Hausenblas; K Heekin; HL Mutchie; S Anton. *J integrative med*, **2015,** 13(4), 231-40.
[38] H Gandomi Nasrabadi; L Azami Sarokelaei; A Misaghi; S Abbaszadeh; N Shariatifar; N Tayyar Hashtjin. *J Med Plants*, **2012,** 2(42), 189-196.
[39] SD Jastaniah. *Life Sci J*, **2014,** 11(8), 78-84.
[40] M Mashreghi; S Shayestehpour. Jundishapur *J Microb*, **2016,** 2011(5, Suppl).
[41] G Acar; NM Dogan; ME Duru; I Kıvrak. *African Microbiol Res*, **2010,** 4(11), 1154-1161.
[42] CJ Zheng; L Li; WH Ma; T Han; LP Qin. *Pharmaceutical biol* **2011,** 49(7), 756-63.
[43] H Motamedi; E Darabpour; M Gholipour; SM Seyyed Nejad. *J Zhejiang Univ Science B*, **2010,** 11(7), 506-11.
[44] H Vahidi; M Kamalinejad; N Sedaghati. *Iran J Pharm Res*, **2010,** 20, 33-35.
[45] AM Mudasir; TS Rajesh; MV Rameashkannan; AP Riyaz; BR Muthu. *Adv Biotech*, **2011**, 2(11), 35-38.
[46] H Vahidi; M Erfan; M Kamalinejad; M Avadi; D Vafaee. Antimicrobial effect of various fractions of Crocus Sativus L. 's stigma on oral pathogen microorganisms. Shahid Beheshti University. Pharmacology School, **2007.**
[47] CaLS Institute, Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; Approved Standard—Ninth Edition. **2012**.
[48] LC Galvao; VF Furletti; SM Bersan; MG da Cunha; AL Ruiz; JE de Carvalho; A Sartoratto; VL Rehder; GM Figueira; MC Teixeira Duarte; M Ikegaki; SM de Alencar; PL Rosalen. Evidence-based complementary and alternative medicine : eCAM 2012, **2012**, 751435.
[49] S Bathaei; M Afshari; A Bou Alhasani; B Etemadikia; A Mousavi Movahedi. Iran J Med Aromat Plant. **2006**, 22(2), 85-97.
[50] R Razzaghi; R Noorbakhsh; A Hemmati Kakhaki; M Saberi Najafi In Evaluation of antimicrobial effects of Crocus sativus stigmas constituents, Proceedings of 3rd National Symposium on Saffron. Mashhad-Iran, **2003**, 239-44.
[51] C De Monte; B Bizzarri; MC Gidaro; S Carradori; A Mollica; G Luisi; A Granese; S Alcaro; G Costa; N Basilico; S Parapini; MM Scaltrito; C Masia; F Sisto. *J. Enzyme Inhib Med Chem*, **2015**, 30(6), 1027-33.
[52] C Pintado; A de Miguel; O Acevedo; L Nozal; JL Novella; R Rotger. *Food Control* **2011**, 22(3), 638-642.
[53] E Lobo. CIBTech *J Microbiol* **2013,** 2(2), 45-53.